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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A01N 37/18, 43/50, C07H 21/04, C12P 21/04, 21/06, G01N 33/48

A1

(11) International Publication Number: WO 97/13410

(43) International Publication Date: 17 April 1997 (17.04.97)

(21) International Application Number: PCT/US96/16237

(22) International Filing Date: 11 October 1996 (11.10.96)

(30) Priority Data:
60/005,431 13 October 1995 (13.10.95) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/728,747 (CON)
Filed on 11 October 1996 (11.10.96)

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HYBRID MOLECULES CONTAINING AMIDATED POLYPEPTIDE BINDING LIGANDS

(57) Abstract

Disclosed are hybrid molecules containing a first part, a second part and third part connected by covalent bonds. The first part contains a chemical entity to be introduced into an animal cell. The second part contains a polypeptide effective to translocate the chemical entity across the cytoplasmic membrane and into the cytoplasm of the animal cell. The third part contains a C-terminal amidated polypeptide ligand effective to cause the hybrid molecule to bind to the animal cell. The amidated hybrid molecules exhibit greater biological activity than non-amidated counterparts. Preferred hybrid molecules are fusion toxins which contain an amidated neuropeptide, e.g., substance P or gastrin releasing peptide. Methods of preparing the hybrid molecules, which in preferred embodiments are prepared enzymatically by peptidlglycine-α-amidating monooxygenase, are also disclosed. Further disclosed are methods of using the hybrid molecules for medical purposes, such as in the fields of diagnostics and therapeutics.

Claims:

1. A hybrid molecule, comprising a first part, a second part and a third part connected by covalent bonds, wherein:

said first part comprises a chemical entity to be introduced into a cell of an animal;

said second part comprises a polypeptide effective to translocate said first part across the cytoplasmic membrane and into the cytoplasm of the cell; and

said third part comprises a C-terminal amidated polypeptide ligand effective to cause said hybrid molecule to bind to the cell.

2. The hybrid molecule of claim 1, wherein said third part is a neuropeptide.

3. The hybrid molecule of claim 2, wherein said neuropeptide is substance P.

4. The hybrid molecule of claim 2, wherein said neuropeptide is Neurokinin A.

5. The hybrid molecule of claim 2, wherein said neuropeptide is Neurokinin B.

6. The hybrid molecule of claim 1, wherein said third part is gonadotropin releasing hormone.

7. The hybrid molecule of claim 1, wherein said third part is corticotropin releasing hormone.

8. The hybrid molecule of claim 1, wherein said third part is calcitonin gene related peptide.

9. The hybrid molecule of claim 1, wherein said third part is growth hormone releasing hormone.

10. The hybrid molecule of claim 1, wherein said third part is thyrotropin releasing hormone.

11. The hybrid molecule of claim 1, wherein said third part is vasopressin.

12. The hybrid molecule of claim 1, wherein said third part is vasoactive intestinal peptide.

13. The hybrid molecule of claim 1, wherein said third part is gastrin releasing peptide.

14. The hybrid molecule of claim 1, wherein said second part comprises a portion of the translocation domain of a naturally occurring toxin.

15. The hybrid molecule of claim 1, wherein said second part comprises a portion of the translocation domain of diphtheria toxin.

16. The hybrid molecule of claim 1, wherein said second part comprises a portion of the translocation domain of Pseudomonas exotoxin.

17. The hybrid molecule of claim 1, wherein said first part comprises a detectable label.

18. The hybrid molecule of claim 1, wherein said first part comprises a nucleic acid.

19. The hybrid molecule of claim 18, wherein said nucleic acid encodes a polypeptide of interest.

20. The hybrid molecule of claim 1, wherein said first part comprises a polypeptide.

21. The hybrid molecule of claim 1, wherein said first part comprises an enzymatically active portion of an enzyme.

22. The hybrid molecule of claim 20, wherein said enzyme is a toxin.

23. The hybrid molecule of claim 22, wherein said toxin is diphtheria toxin.

24. The hybrid molecule of claim 22, wherein said toxin is Shiga toxin.

25. The hybrid molecule of claim 22, wherein said toxin is Shiga-like toxin.

26. The hybrid molecule of claim 22, wherein said toxin is ricin toxin.

27. The hybrid molecule of claim 22, wherein said toxin is Pseudomonas exotoxin.

28. The hybrid molecule of claim 1, wherein the covalent bonds connecting said first and second, and said second and third parts are ach peptide bonds.

29. The hybrid molecule of claim 28, which is a recombinant protein.

30. A fusion toxin, comprising from N-terminus to C-terminus, (a) a polypeptide toxic moiety, (b) a polypeptide capable of translocating said toxic moiety across the cytoplasmic membrane and into the cytosol of a target cell of an animal, and (c) a C-terminal amidated polypeptide ligand effective to cause said fusion toxin to bind to said target cell.

31. A fusion toxin, comprising from N-terminus to C-terminus, fragment A of native diphtheria toxin, or an enzymatically active fragment thereof, peptidically linked to a portion of fragment B of native diphtheria toxin effective to translocate said fragment A across the cell membrane and into the cytosol of a target cell of an animal, peptidically linked to a C-terminal amidated polypeptide ligand effective to cause said fusion toxin to bind to said target cell.

32. The fusion toxin of claim 31, wherein the peptidic linkage between said fragment A and said portion of fragment B of diphtheria toxin comprises the l_1 cleavage domain of native diphtheria toxin.

33. The fusion toxin of claim 31, wherein said fragment A and said portion of fragment B of diphtheria toxin together comprise DAB_{389}.

34. The fusion toxin of claim 31, wherein said C-terminal amidated polypeptide ligand is substance P.

35. The fusion toxin of claim 31, wherein said C-terminal amidated polypeptide ligand is gastrin releasing peptide.

36. The fusion toxin of claim 31, which is $DAB_{389}SP$.

37. The fusion toxin of claim 31, which is $DAB_{389}GRP$.

38. A hybrid molecule comprising a polypeptide capable of translocating a chemical entity across the cytoplasmic membrane and into the cytoplasm

of an animal cell, linked via a covalent bond to a C-terminal amidated polypeptide ligand.

39. The hybrid molecule of claim 38, wherein said covalent bond linking said translocation polypeptide and said amidated polypeptide is a peptide bond.

40. A recombinant DNA molecule encoding a hybrid protein, wherein said protein comprises a first part comprising a polypeptide entity to be introduced into a cell of an animal, a second part comprising a polypeptide effective to translocate said third part across the cytoplasmic membrane and into the cytoplasm of the cell, and a third part comprising a polypeptide ligand extended at its C-terminus by a glycine residue.

41. A recombinant DNA molecule encoding the hybrid protein DAB_{389}-substance P-Gly.

42. An isolated and purified recombinant DNA molecule encoding substance P-Gly.

43. A recombinant DNA molecule encoding the hybrid protein DAB_{389}-GRP-Gly.

44. An isolated and purified molecule encoding GRP-Gly.

45. A recombinant DNA molecule encoding a hybrid molecule comprising a polypeptide capable of translocating a chemical entity across the cytoplasmic membrane and into the cytosol of an animal cell, peptidically linked to a C-terminal glycine-extended polypeptide ligand.

46. A method of preparing a hybrid molecule comprising, from N-terminus to C-terminus, a chemical entity, a translocating polypeptide, and a C-terminal amidated polypeptide ligand effective to cause said hybrid molecule to bind to a target cell of an animal, comprising the steps of:

providing a C-terminal glycine-extended precursor of said hybrid mol cule, or a portion thereof containing said polypeptide ligand; and